filamentous structure at the very tip of sidearm as a newly named "antenna", which is comprised of two anti-parallel coiled-coil structures and crucial for dynein-binding. Furthermore, the composition of the globular head which is important for microtubule-binding is also renewed as the complex of the two discontinuous regions. These findings provide the clues for understanding dynactin functions and its molecular mechanism, especially as a regulator of dynein.

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Slow Dynein E with No Retardation Effects: Implication of Indirect Cooperation with Fast Dynein C

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We highly purified the *Chlamydomonas* inner-arm dynein "e" and "c," which reside in a pairwise fashion along the peripheral doublet microtubules. Electron microscopic observations and single particle analysis showed that the head domains of these two dyneins were similar while the tail domain of dynein e was short and bent in contrast to that of dynein c. The ATPase activities, both basal and microtubule-stimulated, of dynein e ($k_{cat} = 0.27\ s^{-1}$ and $k_{cat,MT} = 1.09\ s^{-1}$, respectively) were lower than those of dynein c ($k_{cat} = 1.75\ s^{-1}$ and $k_{cat,MT} = 2.03\ s^{-1}$, respectively). From in vitro motility assays, microtubule translocation by dynein e was found to be slow ($V = 1.2 \pm 0.1$ μm/s) and appeared independent of the surface density, whereas that by dynein c was very fast ($V_{max} = 15.8 \pm 1.5$ μm/s) and highly sensitive to the decrease in the surface density ($V_{min} = 2.2 \pm 0.7$ μm/s). Dynein e was expected to be a processive motor, judged from the microtubule landing assay. To obtain insight into the *in vivo* roles of dynein e, we measured the sliding velocity of microtubules driven by a mixture of dyneins e and c of various ratios. The microtubule translocation by the "fast" dynein c became even faster in the presence of the "slow" dynein e, suggesting that dynein e would not retard the microtubule translocation by a fast dynein. In flagella, dynein e may hold adjacent microtubules for the sake of dynein c's power stroke. Moreover, dynein e may be recruited into the flagellar motion when the local velocity of microtubule sliding is not high.

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Direct Measurement of the Pressure Generated by a 1D Protein Gas Confined within Microtubule Overlaps

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The integrity of the mitotic spindle during anaphase is facilitated by antiparallel microtubule-microtubule overlaps in the spindle midzone. Within these overlaps, motor proteins (e.g. kinesin-5, kinesin-14) as well as passive, non-enzymatic microtubule crosslinkers (e.g. from the MAP65 family) localize and influence the mechanical stability of the overlaps. Here, we show in vitro that the diffusible microtubule crosslinker Ase1, a member of the MAP65 family, can slow down and halt the shortening of microtubule-microtubule overlaps driven by the kinesin-14 Ncd. Using mathematical modeling we show that Ase1 confined in a microtubule overlap behaves like a 1D gas upon compression, i.e. producing an entropic force opposing the compression. Direct measurement of the entropic force by optical tweezers yielded values in the pN-range, comparable to the forces produced by motor proteins present in the spindle midzone. We hypothesize that entropic pressure may be a general mechanism of force production in biological systems.

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Order from Disorder: The Intrinsically Disordered Protein Tau Facilitates Higher-Order Assembly of Microtubules

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Neurofibrillary tangles, the hallmark intracellular symptom of Alzheimer's disease, are aggregations of hyperphosphorylated tau. In its non-diseased state, the microtubule-associated protein tau, a neuronal polyampholyte, promotes assembly of microtubules and serves other critical cell functions. However, characterizing both diseased and non-diseased states have been difficult. Tau is an intrinsically disordered protein, precluding protein crystallography of its structure. Even studying the effects of tau on microtubules is complicated due to the dynamic instability, the cyclic growth and depolymerization of microtubules. While the drug taxol is often used to stabilize microtubules, there is burgeoning evidence that taxol affects microtubule structure and assembly (M.C. Choi et al., BioPhys J., 2009, 97, 519-527).
Solution x-ray diffraction, optical microscopy, and EM were used to study the structure of microtubule/tau mixtures near physiological conditions. Surprisingly, in the presence of tau, the microtubules organized into an open bundled structure with a large microtubule-wall-to-wall spacing (approximately 20 to 40 nm), much larger than expected from polyampholyte theory. The bundle lattice parameter was modulated by differences in the projection domain, a little understood segment of tau that gives rise to the different N-termini of tau isoforms. To understand the functional structure of tau, constructs with deleted domains were created to see how specific regions (or lack thereof) affected the packing behavior of the microtubules. It was determined that certain regions of tau are not necessary for bundling (in conflict with existing models), but their presence might give insight into tau's physiological role in the neuron and how it might evolve into its diseased state.

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Simulated Cytoskeletal Collapse via Tau Degradation in Late State Alzheimer's Disease

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Amyloid-beta aggregates initiate Alzheimer's disease, and downstream trigger degradation of tau proteins that act as microtubule bundle stabilizers and mechanical spacers. Currently it is unclear which of tau cutting by proteases, tau phosphorylation, or tau aggregation are responsible for cytoskeleton degradation. We present a coarse grained two-dimensional mechanical model for the microtubule-tau bundles in neuronal axons which includes (i) taus modeled as entropic springs between microtubules, (ii) a possible depletion force due to phosphorylated taus between microtubules, and (iii) removal of taus from the bundles due to phosphorylation. We equilibrate upon tau removal using active damped molecular dynamics and measure the bundle's radius of gyration as tau occupation probability falls to zero. In the absence of the depletion force, the microtubule bundles lose rigidity at about 60% tau occupancy, in agreement with standard percolation theory results. With the attractive depletion force, spring removal leads to first order collapse of the bundles at 60% tau occupancy for physiologically realizable conditions. This collapse may be reflected in reduced white matter volume observed via MRI studies of Alzheimer's progression, and suggest mechanical measurements on cultured neurons to test our results. Future work will seek to explore the effects of alternate mechanisms on the bundle radius and expand the model into three dimensions. Supported by NSF grant DMR-1207624.

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No through Traffic: Modeling Tau Inhibition of Kinesin Motility

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While the microtubule associated protein Tau is known to be a potent inhibitor of kinesin-1 motility, our recent experimental work has demonstrated that kinesin-2 is insensitive to the presence of Tau under identical conditions. Furthermore, we have shown that sensitivity to Tau is dependent on the contour length of kinesin's neck-linker, which is longer in kinesin-2 than kinesin-1. We hypothesize that kinesin-2's longer neck-linker allows it to bypass obstacles on the microtubule surface by side-stepping to an adjacent protofilament, while kinesin-1, with its shorter neck-linker, is limited to tracking a single protofilament and therefore is susceptible to disruption of motility by microtubule-associated proteins, such as Tau. However, kinesin-2's longer neck-linker has also been shown to account for its decreased processive run length along the microtubule surface relative to kinesin-1, which may also contribute to kinesin-2's insensitivity to Tau. To distinguish between these possibilities, we have developed a simple stochastic model of kinesin motility in the presence of Tau, with Tau behaving as a point-wise steric inhibitor. When calibrated with the well-characterized kinesin-1 behavior, our simulations predict a resolvable inhibition of kinesin-2 motility, an observation that is not supported by our experimental results. Thus, the observed insensitivity of kinesin-2 to Tau inhibition is directly related to its ability to navigate around obstacles on the microtubule surface and not merely an artifact of its decreased processive run length.

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Interplay between Velocity and Travel Distance of Kinesin-Based Transport in the Presence of Tau

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Although the disease-relevant microtubule-associated protein tau is known to severely inhibit kinesin-based transport in vitro, potential mechanisms for